APPENDIX B

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Data Supplement for CHOP Disclosure #13, October 30th, 2001

Experiments have been carried out demonstrating proof of concept and feasibility for reverse gene therapy. A long QT syndrome mutation, Q9e-MiRP1 (Figure 1), was cloned into a bicistronic expression vector (CMV promoter), with a FLAG peptide sequence at the C-terminus, and a neomycin resistance region. The wild-type MiRP1 sequence was cloned into the identical vector for comparisons. Q9E has been reported to cause the long QT syndrome, and life-threatening arrhythmias following clarithromycin exposure (Cell 97:175, 1999). Therefore, when used locally in site specific reverse gene therapy, Q9E is quiescent, unless clarithromycin is administered, hypothetically resulting in slowing of the conduction in rapid re-entrant arrhythmia circuits. Thus, in practice a gene delivery implant in the heart loaded with vectors for Q9E-MiRP1 could result in overexpression of this channel in a desired region, that then later could respond selectively (the rest of the heart would not respond) to clarithromycin for clinically indicated anti-arrhythmia therapy.

Figure 2: Illustrates successful transfection using lipofection with G418 sorting of HEK293 cells, demonstrating vector expression (via green fluorescent protein), and membrane localization of either the wild-type channel or Q9E, as shown by FLAG-rhodamine immunohistochemistry per Laser confocal microscopy. Patch clamp data with current vs. voltage clamp plots are shown on the right side of the Figure, demonstrating dramatically diminished Ikr currents with 1.5mM clarithromycin administration, thereby documenting the antibiotic selective effect.

Figure 3: Demonstrates successful transfection with GFP & MiRP1 expression (either wild type or Q9E) in mesenchymal stem cells. Laser confocal microscopy (Rhodamine-immunohistochemistry-anti-FLAG) documents cell membrane localization of either the wild-type or Q9E MiRP channel. These data demonstrate that this approach could be used to modify stem cells, which could then be used in actual cardiac regeneration transplants, to provide regions of the myocardium which would be responsive to this reverse gene therapy approach, re. selective regional responsiveness to clarithromycin as above.

Figure 4: Results of a five animal study demonstrating Q9E-MiRP1 (or wild-type vector) pig atrial myocardial gene expression one week following vector administration using DNA antibody micelles (50μg DNA per 100 microliter injection site) showning GFP positive cells, as documented with immunohistochemistry. H& E studies demonstrate minimal to no inflammation.

Figure 5: Morphometry studies of the pig right atrial samples (per Figure 4) indicated that 15% of cardiac myocytes were transfected with only 50μg of DNA in the injected region, with the same level of transfection per morphometry studies for either the wild-type or Q9E mutations. Laser confocal microscopy of frozen sections of atrial myocardium (Rhodamine-immunohistochemistry-anti-FLAG) demonstrates cell membrane localization of either the wild-type or Q9E MiRP channel.

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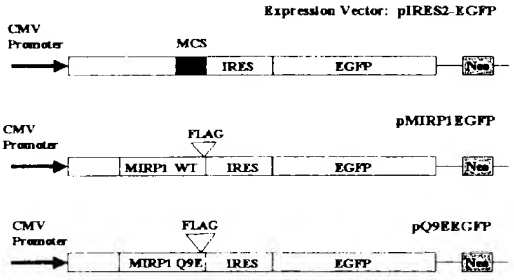
Figure 1

Expression Constructs



MIRP1 Point Mutations

TM Domain

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Rat MiRP1:      1   MTTLAWLTQTLEDAFKKVFITYMDSWRRWTTAEQQALQARVDAENFYYVILYLAMVMIGMF 60
                    M+TL+W TQTLED F+++FITYMD+WR+WTTAEQ+ALQA+VDAENFYYVILYLAMVMIGMF
Human MiRP1     1   MSTLSWFTQTLEDVFRRIFITYMDNWRQWTTAEQEALQAKVDAENFYYVILYLAMVMIGMF 60
                                Q9E                                  M54T  I57T
                                T8A
Rat MiRp1:     61   AFIVVAILVSTVKSKRREHSQDFYHQYIVEDWQQKYRSQILHLEDSKATIHENLGATGFT 120
                    +FI+VAILVSTVKSKRREHS DFYHQYIVEDWQ+KY+SQIL+LE+SKATIHEN+GA GF
Human MiRP1:    61   SFIIVAILVSTVKSKRREHSNDFYHQYIVEDWQEKYKSQILNLEESKATIHENIGAAGFK 120
                                                                    A116V
Rat MiRP1:     121   VSP 123
                     +SP
Human MiRP1    121   MSP 123
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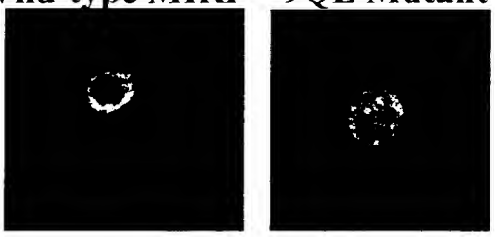
MiRP1 Mutants	Drugs induced arrhythmia (increase channel blockage)	References
Q9E	clarithromycin	Cell 97:175,1999
M54T	procainamide	PNAS 97:10613,2000
I57T	oxatomide	PNAS *ibid.*
T8A	sulfamethoxazole	PNAS *ibid.*
A116V	quinidine	PNAS *ibid.*

Figure 2

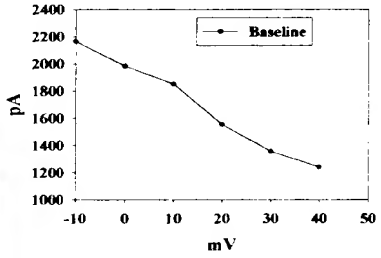
HEK 293 Cells

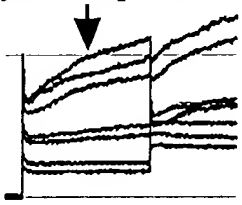
Wild-type MIRP **9QE Mutant**

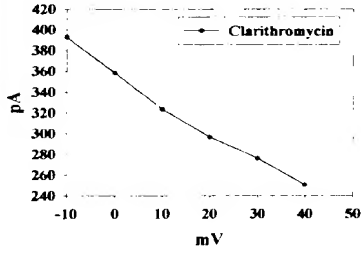


FITC=GFP,
Rhodamine=anti-FLAG-[MIRP]

9QE Cell Electrophysiology Data Showing Clarithromycin Response







Arrows indicate end of activation pulse

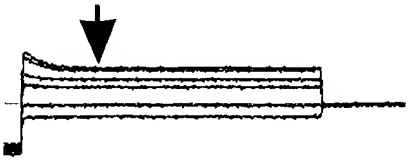


Figure 3

Rat Mesenchymal Stem Cells

Wild-type MIRP

9QE Mutant

FITC=GFP

Rhodamine=anti-FLAG-[MIRP]

Figure 4: Pig Myocardial Results

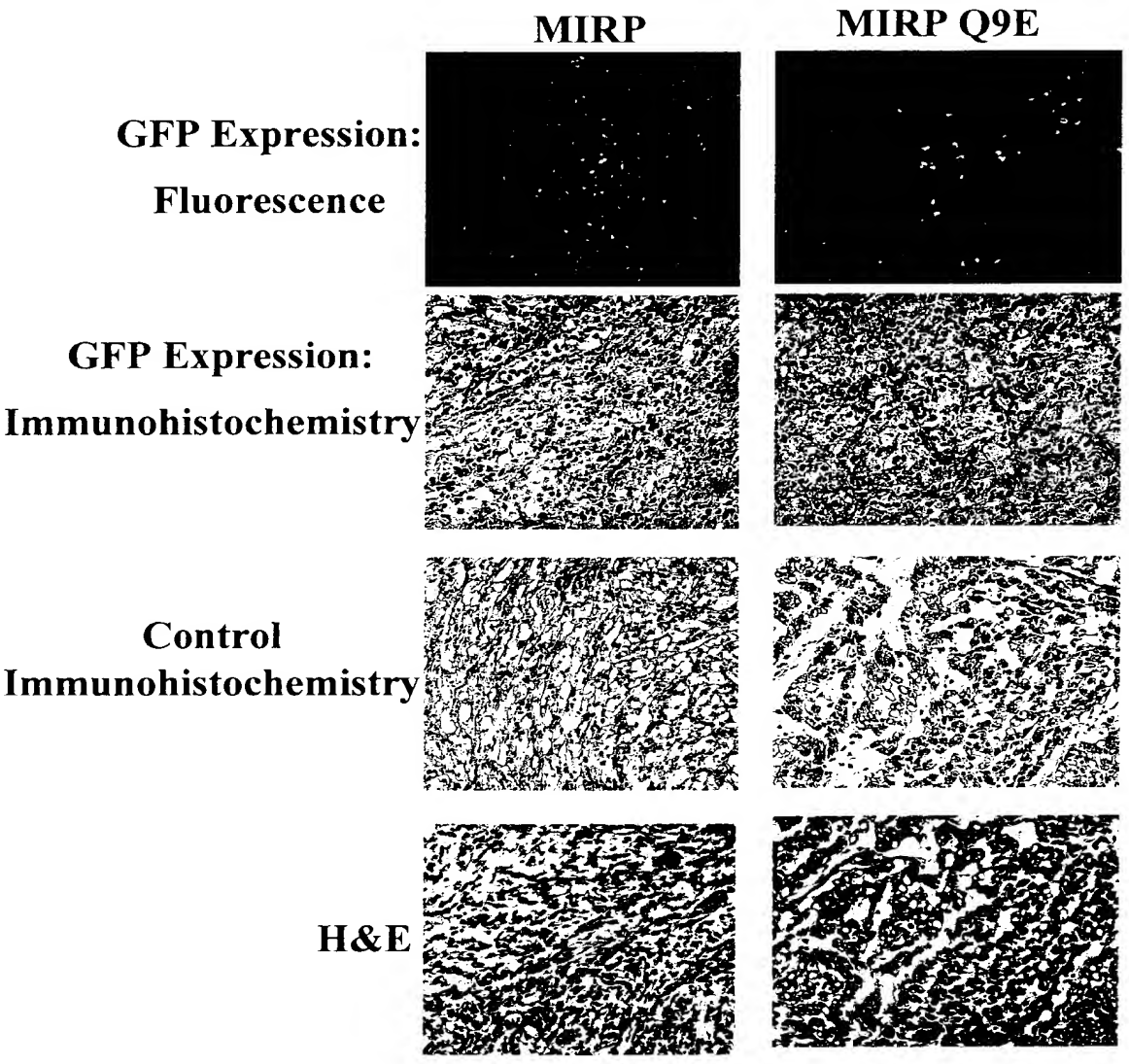
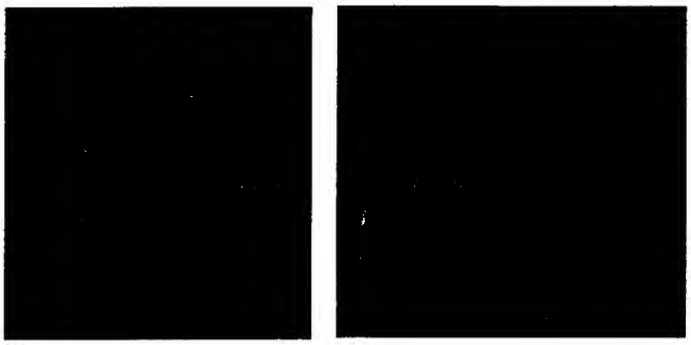


Figure 5: Pig Myocardial Results

MIRP **MIRP Q9E**



Blue=DAPI
FITC=GFP
Rhodamine=anti-FLAG-[MIRP]